

Wildlife As Sentinels of Human Health Effects in the Great Lakes–St. Lawrence Basin

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There is no existing formal, long-term program for gathering evidence of the incidence and severity of the health effects of toxic substances in wildlife. However, research-based studies of bald eagles, herring gulls, night herons, tree swallows, snapping turtles, mink, and beluga over the past 30 years have revealed a broad spectrum of health effects in the Great Lakes–St. Lawrence basin including thyroid and other endocrine disorders, metabolic diseases, altered immune function, reproductive impairment, developmental toxicity, genotoxicity, and cancer. These effects occurred most often and were most severe in the most contaminated sites (Green Bay, Saginaw Bay, Lake Ontario, the St. Lawrence estuary, and more recently, Lake Erie), some of which are International Joint Commission–designated Areas of Concern (AOCs). In all cases, a strong argument can be made for an environmental etiology, and in many cases for the involvement of persistent organic pollutants, particularly polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins, and polycyclic aromatic hydrocarbons. For some, the association with particular contaminants is consistent with controlled studies, and in some, dose–response relationships were documented. The biologic significance of these health impairments to the affected species is currently unclear, but they resemble those observed with increased incidence in human subpopulations in one or more AOCs. Formalizing health effects monitoring of sentinel wildlife species by the parties to the Canada–USA Great Lakes Water Quality Agreement is required. This would facilitate the optimal use of sentinel wildlife health data in a larger, epidemiologic weight-of-evidence context upon which to base decisions and policies regarding the effects of chemical exposures on human populations. *Key words:* beluga, biomarkers, developmental toxicity, effects monitoring, fish-eating birds, genotoxicity, Great Lakes, human health, immune response, metabolic diseases, sentinel species, thyroid, tumors, turtles. — *Environ Health Perspect* 109(suppl 6):853–861 (2001). <http://ehpnet1.niehs.nih.gov/docs/2001/suppl-6/853-861fox/abstract.html>

Animals have long served as monitors and sentinels of environmental hazards. By Roman times, coal miners were aware of the great sensitivity of birds to the poisonous action of carbon monoxide and took them underground to warn of eminent hazard. As recently as 1916, the U.S. Bureau of Mines recommended canaries in particular for this purpose (1). In 1962, Rachel Carson wrote, “our fate is connected with the animals” (2). The wildlife–human connection was an integral part of the discussions in three of six Wingspread Conferences organized by Theo Colborn between 1991 and 1996 (3–5) that focused upon the effects of environmental contaminants on the development and function of the endocrine, reproductive, nervous, and immune systems. Both these visionaries recognized that humans and animals share local environments, air, water, and food chains, and that the molecular, biochemical, and cellular processes responsible for the pathobiologic response to toxic agents are common among most vertebrate species.

While studies of animals under controlled laboratory conditions are very useful for determining dose–response relationships and the molecular mechanisms of toxicity, they tell us nothing about integrated biologic responses to chronic exposure to real-world concentrations of

mixtures of environmental chemicals. Wildlife not only provide information on the types, characteristics, amounts, and bioavailability of pollutants in an environment, but also provide important information on the interactive effects of these environmental chemicals and the role of other environmental factors in the final toxicologic response. Such data provide the basis for hypothesis generation.

In 1972, the U.S. and Canadian governments signed the Great Lakes Water Quality Agreement (6), which contains provisions for the protection of human health and for monitoring trends in the concentrations and effects of persistent toxic substances. In 1989, the Science Advisory Board to the International Joint Commission (IJC) recommended that the parties to the Canada–U.S. Great Lakes Water Quality Agreement of 1972 use the results of studies on health effects in wildlife populations as a basis for decisions and policies regarding the effects of chemical exposures on human populations, pending the availability of more information on human health.

Here I present the state of knowledge gained from wildlife monitoring and research programs in relation to the types of adverse health outcomes identified in Health Canada’s reports (7) on the 17 Canadian

Areas of Concern (AOCs) and the recent literature. The majority of observations of health effects in individual animals reported herein were made in small samples (<20 individuals), collected on a single day, from a restricted number of locations, only some of which were AOCs. They are a conservative estimate of the incidence and severity of specific adverse health outcomes selected on the basis that they may be linked to environmental contaminants (8).

Assessing the Health of Humans versus Wildlife

In Canada, the births, congenital anomalies, hospital admissions, diagnosed cancers, and causes of death of humans are recorded. Populations at risk can be questioned or examined when required. Many of these data are archived in publicly funded centralized databases, and data for defined geographic areas can be retrieved for epidemiologic studies. Such data were used by Health Canada to prepare the reports on the health and well-being of people living in communities within and around the 17 AOCs (7).

In contrast, the births of free-living wildlife go unrecorded. No clinical assessment of their vitality, weight, sex, freedom from deformities, etc., is made or recorded. Diseased, reproductively incapable, or otherwise dysfunctional individuals in wildlife populations do not visit doctors or hospitals for treatment of their complaints. If, or when, such individuals die, there is no record. Very few are found and thoroughly autopsied, and most of those represent highly visible, localized epidemics (9). Hence, morbidity and mortality are rarely detected and grossly underestimated in wildlife populations. Therefore we greatly underestimate the occurrence, prevalence, and severity of health effects in any wildlife population we study; the individuals we see or study are the survivors.

Wildlife biologists consider disease as

“any impairment that interferes with or modifies the performance of normal

This article is based on a presentation at the Workshop on Methodologies for Community Health Assessment in Areas of Concern held 4–5 October 2000 in Windsor, Ontario, Canada.

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Received 5 February 2001; accepted 11 April 2001.

functions, including responses to environmental factors such as nutrition, toxicants, climate, infectious agents, inherited or congenital defects, or combinations of these factors" (9).

Their focus is on functional impairment and recognition of the multifactorial nature of disease rather than on death.

Fish-eating wildlife, like humans, are terminal members of the fish-eating guild of the Great Lakes ecosystem. Unlike most human populations residing in the Great Lakes basin, their diet is composed almost entirely of fish and components of the Great Lakes food chain. Health of humans is also affected by occupation, life style, and heredity. In the case of wildlife, occupation reflects the ecologic niche of a species, and life style is reflected in prey choice. In contrast to modern human populations, populations of free-living wildlife are panmictic and unaffected by ethnicity, religious practices, and long-distance immigration, thereby reducing the relative importance of heredity in wildlife disease. This shifts the focus of the wildlife biologist to diet and the environment.

The process of environmental disease is a continuum from the onset of exposure to the stressor(s) through precursor or compensatory states (impairments) that precede the clinical onset of sicknesses, or dysfunctions that culminate in irreversible effects or death. The latter lead to population-level consequences (alterations in abundance and distribution, age and sex ratios, and genetic diversity) and potential extinction of the local population. Generally, for the sake of economy and rapidity, wildlife investigations focus on individuals in their natural environment in the belief that the well-being of the population can be assessed at any point in time by determining the pathophysiologic and functional status of its individual members. Monitoring for the underlying impairment of physiologic (molecular, biochemical, and cellular) and behavioral responses is believed to provide an early warning (before the onset of disability and frank disease), an understanding of the mechanisms by which health is impaired, and a basis for the development of cause-effect linkages (10–12). However, demographic measures (numbers, survival and recruitment rates, age structure, sex ratios) are also needed to *a*) confirm and build linkages with biochemical and functional measures and develop predictive modeling capacity, *b*) identify populations that are detrimentally affected, and *c*) ensure that we do not miss the adverse cumulative impacts of multiple stressors (13). Currently, demographic data are collected only for those species harvested or considered to be at risk.

Potential Wildlife Sentinels for the Great Lakes–St. Lawrence Basin

Wildlife are highly mobile and generally widely dispersed. Therefore, health assessments are limited to those stages of the life cycle when individuals are relatively immobile (embryos of birds and turtles, flightless young) and aggregated (adult and prefledgling colony-nesting birds, nesting turtles). Each life stage differs in the timing, type, and degree of exposure to contaminants (14). Unlike the mammalian fetus, the eggs of birds, turtles, and other oviparous vertebrates are an isolated and independent metabolic system (14). Lipophilic persistent bioaccumulative contaminants such as 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) are bioconcentrated in egg-yolk lipoproteins by orders of magnitude over the ambient concentrations in the female's diet and are part of the embryo's milieu from fertilization to hatching (14). Populations of some species in the Great Lakes–St. Lawrence ecosystem are such that only addled eggs and prefledgling blood and feathers (bald eagle, Caspian tern) or carcasses of trapped (mink and otter) or stranded (beluga) individuals can be used for health assessments, limiting the biomarkers and functions that can be measured.

Wildlife species differ in their distribution, and their migratory, food, and reproductive habits, which in turn influence their ease of study and value as sentinels. The mobility of fish-eating birds makes them regional or lakewide indicators. The bald eagle (*Haliaeetus leucocephalus*) is a long-lived, year-round resident of the entire Great Lakes Basin, and like humans, a tertiary predator in the Great Lakes food web (15). As the symbol of the United States, this species is one of the most studied in North America. It was the first species in which toxicologic effects of persistent toxic substances were observed (15), and as such, has been repeatedly recommended by the IJC as an ecosystem monitor of Great Lakes water quality. It has recently been adopted as the primary wildlife biosentinel by the Michigan Department of Environmental Quality for their Clean Michigan Initiative. This is the only wildlife species in which we can count and monitor the reproductive outcomes of all the pairs breeding on Great Lakes shorelines. The breeding population of this solitary nester has been censused aerially since the early 1960s, and data exist for many breeding areas for over 35 years (15). Efforts are currently under way to develop a battery of blood-based biomarkers to assess the health of 6- to 12-week-old nestlings. The herring gull

(*Larus argentatus*) is also a year-round resident and an opportunistic fish-eater that has been extensively studied throughout the Great Lakes since the mid-1960s (16). In 1974, the Canadian Wildlife Service initiated a program to monitor persistent lipophilic contaminants in the eggs of this colonial species in the Great Lakes (17). This ongoing program has identified new contaminants and their sources, geographic and temporal trends, factors that regulate contaminant bioavailability, dynamics, and accumulation, and provided a measure of the effectiveness of remedial measures (16,17). Many aspects of the health of adults of this long-lived species have been assessed throughout the basin over the past 25 years (18), and more recently, a suite of biomarkers has been applied to prefledglings (19). The black-crowned night heron (*Nycticorax nycticorax*) was chosen by the U.S. Fish and Wildlife Service as its estuarine and freshwater sentinel for the nationwide Biomonitoring of Environmental Status and Trends Program. Considerable work has gone into the development of biochemical markers in pipping embryos (20,21), and efforts are currently under way by Canadian researchers to apply some of the biomarkers developed for prefledgling herring gulls to this species. The great blue heron (*Ardea herodias*) has been adopted by the Quebec Region of the Canadian Wildlife Service as a biosentinel for the condition of the St. Lawrence River, using eggs and plasma of prefledglings (22). Both heron species are migratory, colonial, and feed in shoreline wetlands. With the banning of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) in the Great Lakes basin in the 1970s, the population of the double-crested cormorant (*Phalacrocorax auritus*) has increased from a low of less than 100 pairs in 1970 to approximately 50,000 pairs in the 1990s (23). This species captures its prey by underwater pursuit and therefore has greater access to all species of forage fish at all depths. This species has been monitored for developmental abnormalities (24–27) but has not been used extensively for biomarker studies. The population biology and reproductive success of the Caspian tern (*Sterna caspia*) have been extensively studied in Lakes Ontario, Huron, and Michigan (25,28,29). Dead eggs and live young have been assessed for the incidence of developmental abnormalities (25,28), and immune and thyroid function of prefledglings have been assessed in colonies in the lower lakes (19,30).

Three species are particularly effective indicators of local contamination. The snapping turtle (*Chelydra serpentina*) is an omnivorous, very long-lived reptile that commonly inhabits wetlands on Great Lakes shorelines (with the exception of the north shore of Lake Superior) (31). It has a very limited

home range throughout the year. A very successful protocol has been developed for collection and artificial incubation of eggs and assessment of hatchlings and congenital abnormalities (31,32). Efforts should be made to develop methods to assess the growth and a suite of biomarkers in hatchlings, and to collect blood samples from adults for biomarker studies. Mink (*Mustela vison*) and river otter (*Lutra canadensis*) are distributed throughout the Great Lakes basin. Their diet provides an integrated exposure to contaminants in shoreline wetlands and tributaries (33,34). The semi-domesticated ranch mink is the most sensitive of mammalian species to polybrominated biphenyls, hexachlorobenzene, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) evaluated to date (33,35). In addition to salvaging carcasses from trappers for contaminant burden analysis, the abundance (in relation to trapping effort), age structure (36), and morphology of the baculum (37) of the trapped mink and otter populations should be determined. The migratory and short-lived tree swallow (*Tachycineta bicolor*) feeds on emergent insects over wetlands close to its nest (38). The residue content of emergent insects reflects the bioavailability of contaminants in the sediments and the transfer of these contaminants to terrestrial food chains. Considerable effort has been put into assessing the usefulness of a suite of biomarkers in nestlings of this species. Nesting colonies can be created where desired by erecting suitable nestboxes.

The health of beluga whales (*Delphinapterus leucas*) reflects the risks associated with life in the polluted St. Lawrence River and estuary downstream from the Great Lakes. Beluga reach sexual maturity between 5 and 10 years of age, and individuals live as long as 30 years. Calves receive nourishment solely via mother's milk, which is very high in lipids, for a period of 20 months. An epidemiologic study is ongoing of this isolated, endangered population, based on postmortem examinations of stranded individuals. It is assumed that the autopsied animals represent an unbiased sample of the total stranded animals and are representative of the population in terms of causes and extent of mortality (39–41).

Alterations in Endocrine Function

Goiter and Thyroid Economy

The majority of adult herring gulls collected from colonies in the Great Lakes from 1974 to 1983 had enlarged thyroids (goiter) relative to those from a coastal marine reference site (42). Their thyroids were microfollicular and frequently hyperplastic. Collectively, the Lake Erie collections had the greatest thyroid mass, the highest prevalence of epithelial

hyperplasia, and the smallest follicle diameters and epithelial areas of the five lakes. The majority of adult gulls collected from 11 Great Lakes colonies in the early 1990s also suffered from goiter (43), which was most severe in the Lake Erie colonies. However, thyroid mass and the incidence of microfollicular hyperplasia decreased between the 1980s and 1990s (43,44). The gulls collected in the early 1990s were also hypothyroxinemic (43) relative to those from the coastal and freshwater reference sites. Plasma thyroxine concentrations were 71% lower in gulls from Middle Island in Lake Erie. The decreased thyroxine concentrations were associated with *ortho*-substituted PCBs but not with TCDD or TCDD-like PCBs, polycyclic aromatic hydrocarbons (PAHs), DDE, or other organochlorine insecticides (43).

Moccia et al. (45) reported that salmon (*Oncorhynchus* sp.) from Lake Erie had the most severe thyroid pathology and highest goiter frequency of all Great Lakes salmon collected from 1976 to 1977. This interspecies correspondence is consistent with the hypothesis that a forage fish-borne factor was responsible for the thyroid abnormalities in both gulls and salmon. The hypothesis that the tissues of Great Lakes fish contain thyrotoxic agents has been supported by two independent studies in which thyroid dysfunction occurred in rats fed salmon from the Great Lakes but not in controls fed salmon from the Pacific Ocean (46,47). As recently as 1987, the most common thyroid disease in residents of Michigan was endemic goiter not goiters due to iodine deficiency (48).

Hypothalamo–Pituitary–Adrenal Axis

Activation of the hypothalamo–pituitary–adrenal (HPA) axis in response to environmental stressors results in corticosterone production, which triggers important adaptive physiologic changes in intermediary metabolism, growth, and immune function. The basal corticosterone concentrations in plasma of near-term embryos of Great Lakes herring gulls collected from five colonies in 1997 were negatively correlated with the concentrations of PCBs, PCDDs, and PCDFs in their yolk sacs (49).

Adrenal anomalies were highly prevalent in St. Lawrence beluga, including hyperplasia and nodular hyperplasia of the cortex, and cortical cysts filled with cortisol-rich fluid (50). The relative importance of age, stress, and adrenotoxic contaminants in the etiology of these lesions is unknown.

Metabolic Diseases

Exposure to environmental chemicals may result in metabolic derangements that detrimentally alter synthetic or degradative processes or that deplete energy sources.

Alterations in Glucose Metabolism

Normal levels of glucose, the primary energy currency used by most tissues, are the result of a finely balanced system of interactions of hormones originating in the pituitary, pancreas, adrenals, and thyroid. Diabetes is a disorder of glucose metabolism resulting from an absolute or relative deficiency of the pancreatic hormone insulin. Diabetes has been reported in virtually all laboratory and domestic mammals, but is a clinical rarity in birds (51). Birds are also very resistant to the experimental manipulations that cause the onset of this condition in other vertebrates (51,52). Normal fasting blood glucose concentrations in birds are 1.5–3 times those of fasting mammals and are maintained more or less constant regardless of dietary levels or feeding frequency (51). Glucagon is the dominant pancreatic hormone in birds, favoring catabolic or retrieval reactions that assure the adequate availability of energy substrates to sustain a high metabolic rate under stressful and diverse metabolic demands (51). A variety of fish-eating and carnivorous birds have been shown to be glucose-intolerant due to the near absence of glucokinase, the enzyme responsible for hepatic clearance of glucose (53). Adult Great Lakes herring gulls are mildly hyperglycemic relative to reference sites (43). This hyperglycemia is not associated with body condition or stress.

Intermediary Metabolism

The activity of phosphoenolpyruvate carboxykinase (PEPCK) and malic enzyme, two intermediary metabolic enzymes, is regulated, at least in part, by corticosterone released from the HPA axis. PEPCK is a key regulatory enzyme in the gluconeogenic pathway. Malic enzyme is a critical provider of NADH for fatty acid synthesis in birds. The activities of these enzymes in tissues of near-term embryos of Great Lakes herring gulls collected from five colonies in 1997 were low relative to those from the reference site and inversely correlated with the concentrations of PCDDs and PCDFs in their yolk sacs (49).

Wasting

Wasting, characterized by loss of body mass and a voluntary decrease in food intake, is a well-documented symptom of TCDD toxicity in several species of mammals (54,55). In rats, TCDD inhibits PEPCK and several other important gluconeogenic enzymes at doses in the same range as those that induce appetite suppression and mass loss (56–58).

In some tern colonies at Great Lakes sites highly contaminated with PCBs, some chicks fed and attended by their parents grow normally for 10–14 days, then begin to waste, losing their muscle mass. In September

1986, a 100-year flood event mobilized large quantities of sediment-associated PCBs from the Saginaw River. In the years that followed, the reproduction of Caspian terns on the confined disposal facility near the river's mouth collapsed, then slowly recovered (28). No chicks wasted in 1986, but 67% did so in 1987. In the period from 1987 to 1991, 22% of all chicks hatched wasted, and wasting was thought to be responsible for the deaths of 45% of all chicks found dead. Forster's tern chicks on Kidney Island at the mouth of the PCB-contaminated Fox River in Green Bay wasted in 1978, 1987, and 1988 (59–61). In 1988, 42% of the young were found dead near the nest, 57% of which appeared to have wasted (61). Caspian tern and herring gull chicks wasted in Saginaw Bay in 1992 (19).

The observation of decreased basal corticosterone concentrations and PEPCK and malic enzyme activities in near-term herring gull embryos from the Great Lakes collected in 1997 (49) are consistent with the possibility that their basal corticosterone levels are insufficient to induce normal activities of these key intermediary metabolic enzymes. The sensitivity of the HPA axis and these important intermediary metabolic enzymes to PCBs, PCDDs/PCDFs may be pivotal in the alterations in gluconeogenic or lipogenic activity that result in wasting. Wasting was also observed in adult ranch mink fed a diet containing carp (*Cyprinus carpio*) collected from Saginaw Bay carp in 1988 (35).

Porphyria

In susceptible species, chronic dietary exposure to some chlorinated contaminants leads to inhibition of specific enzymes in the heme biosynthetic pathway, resulting in the accumulation of uroporphyrin and other highly carboxylated porphyrins (HCPs). Surveys conducted in 1974 and the early 1980s and 1990s revealed that the livers of varying proportions of adult herring gulls from colonies in Lakes Ontario, Erie, Huron, Michigan, and Superior contained elevated concentrations of HCPs (43,62). Porphyria was most severe in gulls from Green Bay, Saginaw Bay, and Lake Ontario colonies. There was a highly significant decrease in the HCP concentrations in the livers of gulls from Scotch Bonnet Island in Lake Ontario between 1974 and 1991 (44).

In 1972–1973 and the early 1990s, only gull chicks from Lake Ontario colonies had elevated concentrations of HCPs (43,63). In tree swallow nestlings collected from five colonies in southern Ontario in 1993, those from Hamilton Harbour and Toronto Island contained high levels of HCPs relative to those from the reference site (38). The severity of the porphyria in nestling swallows from Lake Ontario in the early 1990s was greater

than that seen in adult or prefledgling Lake Ontario herring gulls; the concentration in the liver of one swallow nestling from Hamilton Harbour is the highest concentration recorded for either species to date. These data suggest that significant quantities of porphyrogenic contaminants in the sediments in these two locations are transferred to terrestrial food chains via emergent insects. Concentrations of HCPs in the livers of hatchling snapping turtles collected in 1991 from a marsh on Lake Ontario were low and did not differ from those from the reference site (32). HCP concentrations were not elevated in the livers of 10 stranded St. Lawrence beluga (64). The degree of porphyria we observed in wildlife species is definitely subclinical in terms of human medicine, and unlikely to be reported.

There was a high degree of correlation between the HCP concentration in a gull's liver and the ability of an extract from that liver to induce porphyrin production in chick embryo hepatocytes *in vitro* (65). There was a high correlation between the HCP concentration in a gull's liver and the PCB concentration in its liver, particularly of the mono-*ortho*-substituted PCB congeners 105 and 118. Other *in vitro* studies conducted in our laboratory suggest that the porphyria in gulls is due to these PCBs and not to DDE or other organochlorine pesticides. The liver concentrations of uroporphyrin in nestling tree swallows collected in the early 1990s were also significantly correlated with the concentration of PCB 118 in the carcass (38). PCBs 105 and 118 have also been relatively potent porphyrinogenic agents in laboratory mice (66).

Functional Immune Response

From 1992 to 1994, immunologic responses were measured in prefledgling herring gull and Caspian tern chicks in colonies distributed across a broad gradient of organochlorine contamination measured in eggs. The phytohemagglutinin (PHA) skin test was used to assess T-lymphocyte function. In both species there was a strong exposure–response relationship between organochlorines [Σ PCBs, TCDD-toxic equivalents (TEQs), and DDE] and suppressed T-cell-mediated immunity (19). Suppression was most severe (30–45%) in colonies in Lake Ontario (1992) and Saginaw Bay (1992–1994) for both species, and in western Lake Erie (1992) for gulls. Both species exhibited differences among sites in B-cell-mediated anti-sheep red blood cell antibody titers, but consistent exposure–response relationships were not observed. In a parallel investigation, thymic atrophy in gull chicks was associated with increasing liver 7-ethoxyresorufin *O*-deethylase (EROD)

activity (67), suggesting that T-cell-mediated immunity was being altered by aryl hydrocarbon (Ah)-receptor agonists (i.e., PCBs and not DDE). In a study of prefledgling Caspian tern chicks from two colonies in Lake Huron from 1997 to 1999, the response to phytohemagglutinin was 42% lower in chicks from Saginaw Bay than in chicks from the North Channel, and there was a strong negative association between the PHA response and plasma PCBs (30). Total antibody titers following immunization with sheep red blood cells were higher in chicks in Saginaw Bay than the North Channel, and were positively associated with plasma PCBs and DDE.

A study by the University of Wisconsin in 1984 found that in Sheboygan (an AOC in American waters), infants exposed to high PCB levels in the womb suffered from more colds, earaches, and the flu (68). A recent Dutch study of 207 healthy mother–infant pairs found that current PCB body burden at 42 months of age was associated with a higher prevalence of recurrent middle-ear infections, and of chicken pox (69). Few wildlife studies have examined the relationship between the prevalence of particular disease and contaminant exposure. When the data of Ludwig et al. (25) were analyzed, a strong correlation was found between the prevalence of severe eye infections in cormorant chicks in colonies on Lakes Michigan and Huron, 1986–1991, and PCB concentrations in eggs from these colonies (18). Stranded beluga from the St. Lawrence have had numerous severe and disseminated infections with a variety of rather mildly pathogenic organisms, and a very high prevalence of tumors consistent with immunosuppression and a decrease in T-cell-mediated immunosurveillance for tumors (39). Similar observations were reported in adult walleye (*Stizostedion vitreum*) from Green Bay in the mid-1990s (70).

Reproductive Impairment

Among the 25 stranded female beluga examined from the St. Lawrence estuary to 1990, 36% suffered from mastitis of one form or another, two had ovarian tumors, and one had an ovarian cyst (39). The rate of pregnancy was drastically low, and evidence suggests that this population is not increasing because of low calf production and/or survival to adulthood (71).

Fish caught in Lakes Michigan and Erie in the early 1960s were implicated in a large number of diet-related reproductive failures in ranch mink (72). Although there was no adult mortality, the number of mated females that gave birth, the number and viability of the kits at birth, and their survival to weaning were all adversely affected. Subsequent investigations reviewed by Gilbertson (73) revealed that the causative agent was lipophilic and not DDT or

dieldrin, but PCBs were strongly implicated. Controlled laboratory studies have shown mink to be very sensitive to the developmental toxicity of specific PCB congeners (73–75). Carp caught as recently as 1988 in Saginaw Bay impaired the reproduction and/or kit survival of ranch mink (35). Concentrations of PCBs in tissues of some wild mink harvested in these areas are equivalent to those measured in ranch mink that experienced reproductive problems (34,76). Densities of mink are thought to be abnormally low in shoreline wetlands in the most contaminated areas of the Great Lakes, although insufficient data on productivity and population trends make population monitoring difficult (34,76).

In the 1950s and 1960s, the reproduction of shoreline-nesting bald eagles and of double-crested cormorants nesting in colonies in all of the Great Lakes began to fail. Both species were extirpated from Lakes Michigan and Ontario, and their numbers greatly reduced in Lakes Superior and Huron, with remnant populations surviving on Lake Erie (15,23,73,77). In the mid-1960s, biologists noted marked decreases in nesting success and precipitous population declines in the breeding population of herring gulls at colonies in upper Green Bay and Grand Traverse Bay in Lake Michigan (73). In all three species the poor reproductive success was associated with DDT/DDE-induced eggshell thinning in excess of 20%, which reduced the ability of the egg to survive the rigors of incubation, and the failure of a large proportion of apparently intact eggs to hatch. The latter was thought to be due to the embryo toxicity of DDT/DDE and, to a lesser extent, dieldrin residues in the egg. In addition, in the herring gull (and probably the bald eagle), there was extensive adult mortality due to the neurotoxicity of these pesticides. Legislative restrictions on the use of DDT in the 1970s have resulted in a reduction in eggshell thinning and a resurgence of the eagle (78) and cormorant (23) populations. With the reduction in eggshell thinning, it became apparent that these and other species of fish-eating birds were suffering from what has been called Great Lakes embryo mortality, edema, and deformity syndrome (GLEMEDS) (59), a suite of effects associated with high egg concentrations of PCBs, PCDDs, and PCDFs (60). GLEMEDS is thought to be equivalent to chick edema disease in PCDD/PCDF-exposed poultry (59). The spectrum of effects seen in embryos and chicks of domestic hens fed a diet containing Saginaw Bay carp collected in 1988 (79) was also similar to that in GLEMEDS.

Developmental Toxicity

The manifestations of developmental toxicity are embryo death, malformation, growth retardation, and functional disorders. These

outcomes often increase in frequency and severity as the exposure increases. Hoffman et al. (21) documented pollutant-related developmental problems in common terns and night herons in colonies in Saginaw and Green Bays in the mid-1980s. They found a significant correlation between femur length:body mass ratio (a measure of growth retardation) and the PCB concentration in eggs from the same colony.

Congenital malformations have been observed in chicks of nine species of fish-eating birds nesting on the Great Lakes in the last 30 years (10,18,73). These include three species of terns, two species of gulls, two species of herons, the double-crested cormorant, and bald eagle. The malformations include abnormal bills; supernumerary, dwarf, or otherwise abnormal appendages; missing or small eyes; and club foot and hip dysplasia and other skeletal abnormalities. Abnormal bills are the most frequently encountered abnormality; they have been reported most often at colonies in Saginaw Bay, Green Bay, and Lake Ontario in cormorants and terns. The highest prevalences of bill abnormalities recorded to date are for cormorants in Green Bay (52/10,000 chicks examined, 1979–1987) (24,27) and bald eagles in Michigan (23/10,000, 1966–1993) (80). The frequency of malformations is considerably higher in dead eggs than in hatched chicks (25). In addition to the above abnormalities, embryos were found with missing jaws, missing skull parts, vertebral abnormalities, exencephaly, anencephaly, hydrocephaly and spina bifida, gastroschisis, and as Siamese twins (25).

In a large study of hatched chicks and live and dead eggs from 37 colonies of cormorants and Caspian terns conducted in the upper Great Lakes from 1986 to 1991, the suite of deformities and abnormalities found (above) was similar to that produced in chickens by exposure to planar PCB and dioxin congeners (25). TCDD-TEQs measured by the H4IIE rat hepatoma EROD bioassay were highly correlated with deformity rates in chicks and dead eggs (25). TCDD-TEQ concentrations were more highly correlated with deformity rates than were total PCB concentrations. Summer et al. (79) reported a dose- and time-dependent increase in terata in embryos and chicks from hens fed a diet containing Saginaw Bay carp caught in 1988; the types of abnormalities are similar to those seen in Great Lakes fish-eating birds.

Developmental abnormalities have also been detected in Great Lakes snapping turtles (31,33). The suite of deformities observed is remarkably similar to that seen in birds, with the addition of curled, bent, twisted, or missing tails (most common abnormality), and abnormalities of the carapace and plastron. Among eggs collected and artificially

incubated from wetland sites on Lakes Ontario and Erie, the St. Lawrence River, and a remote reference site in Algonquin Park, 1986–1991, those collected from sites on Lake Ontario had the highest probability of not hatching and of producing deformed turtles. The eggs from Lake Ontario contained elevated concentrations of PCDDs, PCDFs, and PCBs. Some of the PCDDs and PCDFs were significantly correlated with the occurrences of deformities and unhatched eggs (33). These observations suggest that the deaths and deformities in both developing fish-eating birds and snapping turtles in the Great Lakes in the 1980s and 1990s are caused by compounds that express their effects through a common mechanism of action—activation of the Ah receptor.

High rates of skeletal limb defects were found in mudpuppies (*Necturus maculosus*) collected in the early 1990s from the most contaminated sites on a gradient of contamination in the St. Lawrence River (81). The mudpuppy is a bottom-dwelling aquatic salamander, and these observations suggest that pollutants may interfere with the development or regeneration of limbs in this species (81). One of 45 stranded beluga in the St. Lawrence estuary examined between 1983 and 1990 was a true hermaphrodite (82).

In the 1970s, the operational sex ratios of adult western gulls (*L. occidentalis*) in colonies on the Channel Islands off the coast of southern California, herring gulls in Lakes Ontario and Michigan, and Caspian terns in Lake Michigan were skewed toward females based on the occurrence of supernormal clutches, which are usually the product of female–female pairs (83–85). This was a post-1950s phenomenon, coincident both spatially and temporally with high DDT/DDE contamination. In mammals, *p,p'*-DDE, the abundant ubiquitous stable metabolite of DDT, is an antiandrogen, whereas *o,p*-DDT, a minor and less-persistent component, is estrogenic (86). Coincidentally, the sex ratio of bloaters (*Coregonus hoyi*) harvested by commercial fishermen from Lake Michigan was 97% female in 1967 and decreased to 50% by the late 1980s. The degree of skew was linearly related to tissue *p,p'*-DDE content (87). The relative role of differential mortality and developmental feminization/demasculinization in the creation of the female-biased sex ratio in the fish-eating birds may never be determined.

Feminization of the reproductive tracts of a small sample of pipping herring gull embryos and newly hatched chicks was found in Lake Ontario in the 1970s (83). Abnormalities in gonadal histology have also been found in gull chicks in the late 1990s (88). Measurements of a sexually dimorphic character [ratio of precloacal length to the posterior lobe of the plastron (PPR)] of adult snapping turtles at two

reference sites and three sites on Lakes Ontario and Erie, 1986–1995, revealed decreases in the PPR (feminization) in male turtles from the Great Lakes sites (89). At one Lake Ontario site, the magnitude of this response was such that the PPRs of a significant proportion of the males overlapped with females. The PPR may be analogous to the anogenital distance in rodents, a measure that is very sensitive to the effects of *in utero* exposure to estrogens and antiandrogens (86).

Tumor Incidence and Genotoxicity

The Health Canada reports reveal higher incidence rates in some AOCs for several cancers that have been partly attributed to exposure to pollutants (7). Neoplasms or tumors are infrequent findings in free-ranging wild birds (90). Neoplasms were found in only 9 (0.05%) of approximately 18,000 wild birds on which diagnostic postmortem examinations were conducted at the U.S. National Wildlife Health Center (NWHC), 1975–1981 (91). All were in game birds, and most were potentially of genetic or viral etiology. Only 1 of 2,000 bald eagles processed by the NWHC had a tumor (92). However, in 2 of 112 livers of adult herring gulls collected in 11 Great Lakes colonies in 1991, there were foci of cellular atypia, in which there were increased variations in cell and nuclear size and presence of binucleate cells of potential preneoplastic significance (93). Neoplastic, adenomoid follicular nodules were found in 3 of 120 thyroids of adult herring gulls from 11 Great Lakes colonies in 1991 (94).

Between 1983 and 1994, scientists examined 73 of 175 carcasses of beluga reported stranded on the shorelines of the St. Lawrence estuary. Of these 73 carcasses, 14 (19%) were affected by 15 different malignant tumors (41). Forty percent of the 35 cancer cases reported worldwide in cetaceans occurred in this population. Not only does this whale population appear to have a high prevalence of cancer, but this is particularly marked with regard to adenocarcinomas of the gastrointestinal tract (41). St. Lawrence beluga visit and feed in the Saguenay River, where sediments contain high concentrations of PAHs. Belugas are known to feed on bottom invertebrates and dig in bottom sediments where they could ingest these carcinogenic compounds. Benzo[*a*]pyrene DNA adducts were detected, at concentrations that approached those associated with carcinogenesis in small laboratory mammals, in brain and/or liver tissue of 11 of 12 St. Lawrence belugas (14–29 years of age) but not in the brains or livers of four individuals from the Arctic (40). Analyses of DNA fingerprints from a nonrandom sample of St. Lawrence belugas using minisatellite

probes indicate a reduced level of genetic variation compared to beluga from the Beaufort Sea (95). Higher levels of mean allele frequency in the St. Lawrence population suggest that it is composed of related individuals. The observed differences are consistent with the hypothesis that the lack of recovery of the St. Lawrence population is in part due to reduced genetic variability.

Brown bullheads (*Ameiurus nebulosus*), a bottom-dwelling fish, were sampled in five AOCs in the lower Great Lakes (Hamilton Harbour, Detroit River, Ashtabula River, Cuyahoga River, and Black River) and four nearby, relatively pristine reference sites in 1988–1991, and their hepatic mitochondrial DNA diversity was assessed (96). Mitochondrial DNA is matrilineally inherited. A consistent reduction in haplotype diversity was observed at contaminated sites relative to their nearby reference sites, strongly suggesting that a mechanism such as natural selection, or stochastic events such as population bottlenecks, have reduced genetic variation at all contaminated sites. Tumor frequencies were high (15–55%) in bullheads from the AOCs but were low in the reference sites. In bottom-dwelling fish, liver cancer and lip papillomas are strongly associated with chemical contamination of sediments (97,98). Detailed long-term studies by Baumann and colleagues (99–101) of bullheads in the Black River and its reference site, Old Woman Creek, have shown that prior to 1983, these fish were subjected to an age-selective mortality associated with high prevalences of liver carcinoma. The prevalence of hepatocellular carcinoma was significantly higher in females than males (99). Bullheads in the Black River were subjected to a direct or indirect mortality from hepatic cancer that increased with age and eliminated fish older than 5 years of age from the population. Decreased steel production, followed by closing of the USX coking facility on the Black River in 1983, resulted in a decline in PAH concentrations in the sediments from approximately 1,100 µg/g in 1980 to 4 µg/g in 1987, and a similar decline was seen in bullhead tissues (101). Coincidentally, the incidence of liver cancer declined by 75%; the percentage of 5-year-old fish tripled between 1982 and 1987–1990, and older fish were first found in 1987 (100,101). These observations suggest that the high liver tumor frequency seen in the Black River bullheads was the result of this PAH exposure, and that most fish died by 5 years of age of liver cancer. It is highly probable that this mortality, particularly of females, was a significant contributor to the population bottleneck that reduced genetic diversity in this population.

Between 1992 and 1997, herring gull chicks from colonies located near steel mills

with coking ovens (Hamilton, Detroit, Sault Ste. Marie, East Chicago) inherited statistically significantly more minisatellite mutations than those in colonies from three rural locations in the basin (102). Furthermore, there was a statistically significant correlation between the number of mutations induced and the distance of the colony from the coking ovens. The mutation rate in colonies at urban sites without steel mills (Toronto and Port Colborne) fell midway between the rate in colonies near steel mills and the rural sites. The significance of these germline mutations is not known. However, when fathead minnows (*Pimephales promelas*) were chronically exposed to water containing a nominal concentration of 1.0 µg/kg for 4 months, there was markedly reduced survival of young, two generations removed from the exposure, and an overall reduction of 84% in the reproductive capacity of the F₁ generation (103). This multigeneration effect is most likely the result of a germline mutation.

Behavior and Neurobehavioral Development

Ranch mink that were fed a high proportion of Saginaw Bay carp (equivalent to 2.5 µg/g ΣPCBs) in their diet were listless, nervous when approached, and became anorectic and hyperactive to light and sound (35). Females appeared to have a lack of interest in their kits. At birth, the smaller kits from dams fed diets containing carp were observed to be weak, listless, and less responsive when handled. These behavioral changes are similar to those reported for infants of mothers eating Great Lakes fish (104,105) and rats fed Lake Ontario salmon (106). No assessments of chick behavior have been made in any fish-eating bird. However, the brains of cormorant hatchlings from eastern Lake Ontario in 1991 and deformed eaglets from Michigan and Wisconsin shorelines in 1993 exhibited gross asymmetry associated with TCDD and PCB exposure during embryonic development (107,108).

Altered parental behavior was observed in Lake Ontario herring gulls in the mid-1970s (109) and in Forster's terns in Green Bay in 1983 (60). Similar abnormalities in parental behavior were observed in ring doves (*Streptopelia risoria*) fed an organochlorine mixture (110).

Discussion

One or more sentinel wildlife species in the Great Lakes–St. Lawrence basin are currently or have been afflicted with thyroid and other endocrine disorders, metabolic diseases, altered immune function, reproductive impairment and developmental toxicity, genotoxicity, and cancer. In general, these health effects occurred most often and were

most severe in the most contaminated sites (Green Bay, Saginaw Bay, Lake Ontario, the St. Lawrence estuary, and, more recently, Lake Erie), some of which are AOCs. In all cases, a strong argument can be made for an environmental etiology, and in many cases for the involvement of persistent organic pollutants, particularly PCBs, PCDDs and PAHs. For some, the association with particular contaminants is consistent with controlled studies, and in some, dose-response relationships were documented. Similar health effects have been observed in two species of pelagic fish in the mid-1990s from two AOCs highly contaminated with PCBs. Walleye from Green Bay had a significantly elevated prevalence of hepatic foci of cellular atypia and neoplasms, with higher prevalences in females than males, and suggestions of altered immunocompetence and intermediary metabolism (70). White suckers (*Catostomus commersoni*) from the Sheboygan River had high prevalences of developing nephrons in their kidneys (a compensatory response to nephrotoxicity) as well as hepatic foci of cellular atypia and neoplasms (11a). Although there are some similarities between wildlife health and community health in some of the AOCs, a large amount of work is still required to link the human health outcomes to pollutants and with measures of health in wildlife sentinels.

The biologic significance of the health effects currently seen in one or more species of Great Lakes wildlife is unclear. Research is needed to establish a linkage between the effects observed on biochemical and functional measures in an individual, and alterations in its growth, survival, and reproduction. There is evidence to suggest that eagles nesting on Michigan shorelines and Caspian terns nesting on Saginaw Bay and Green Bay were/are sink populations (29,112). There is little doubt that the recovery of the St. Lawrence estuary beluga population is influenced by contaminants, and it is probable that mink inhabiting the shorelines of the lower Great Lakes suffer from PCB-induced reproductive toxicity. Based on published investigations of belugas (95) and bullheads (96), it is quite likely that contaminants have effected the genetic diversity of other species, the consequences of which may become apparent in the future.

The association of genotoxicity or neoplasia with coking facilities and/or other sources of PAHs in bottom-dwelling fish, beluga, and herring gulls is striking and deserves investigating in the human populations of the Great Lakes basin downwind from such facilities (40,41,100–102). Similarly, the observations of behavioral deficits and abnormalities in rats fed Lake Ontario salmon (106) and in children whose mothers ate Lake Ontario salmon (105) and Lake Michigan fish (104) suggest

that such effects are likely in wild piscivores residing in Green Bay, Saginaw Bay, Lake Ontario, and Lake Erie. However, they go undetected because of a lack of suitable tools for their detection and are likely to be manifest in decreased survival and reproductive fitness (29,35,108,109).

The use of free-living bald eagles, herring gulls, night herons, tree swallows, snapping turtles, mink, and beluga as sentinels over the past 30 years has revealed a broad spectrum of health effects in the Great Lakes–St. Lawrence basin. The variation in mobility, duration of residence, and diet of wildlife residing or feeding in AOCs is analogous to that of human communities within and around AOCs (113). Both wildlife and humans are exposed through multiple media (112,114). There is a need to develop additional assays, particularly those that measure specific functions, as well as indicators of free-radical-mediated damage, neoplasia, and energetic efficiency, and to incorporate them into our diagnostic panel for these sentinel species. According to the National Academy of Science's Committee on Animals as Monitors of Environmental Hazards, a true animal sentinel system is a system in which data on animals exposed to contaminants in the environment are regularly and systematically collected and analyzed to identify potential health hazards to other animals or humans (114). All of the information on wildlife health in the Great Lakes reported here was gathered as a result of academic research or research-based monitoring. Most was short-term, localized, and not systematic, and as such, incapable of providing an accurate or comprehensive picture. Although there are adequate long-term monitoring programs to document gross trends in concentrations of pollutants in the Great Lakes environment, there is no formal existing program in either Canada or the United States for gathering long-term evidence for determining trends in the incidence and severity of the effects of persistent toxic substances in wildlife (115). Formalizing health effects monitoring of one or more sentinel wildlife species by the parties to the Canada–U.S. Great Lakes Water Quality Agreement (6) would facilitate the optimal use of wildlife health data in a larger, epidemiologic weight-of-evidence context upon which to base decisions and policies regarding the effects of chemical exposures on human populations (112,116). Time is of the essence.

REFERENCES AND NOTES

- Burrell GA, Seibert FM. Gases Found in Coal Mines. Miners' Circular 14. Washington, DC: Bureau of Mines, Department of Interior, 1916.
- Carson R. Silent Spring. Boston, MA: Houghton Mifflin Co., 1962.
- Colborn T, Clements C, eds. Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Vol 21: Advances in Modern Technology. Princeton, NJ: Princeton Scientific Publishing, 1992.
- Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Environ Health Perspect 104(suppl 4):807–842 (1996).
- Colborn T, Short P, Gilbertson M, eds. Health Effects of Contemporary-Use Pesticides: The Wildlife/Human Connection. Toxicol Ind Health 15(1–2) (1998).
- Revised Great Lakes Water Quality Agreement of 1978. Agreement, with Annexes and Terms of Reference, between the United States and Canada signed at Ottawa, ON, Canada, 22 November 1978 and Phosphorous Load Reduction Supplement signed 16 October 1983, as amended by Protocol, signed 18 November 1987. Office Consolidation. International Joint Commission United States and Canada, Windsor, ON, Canada, September 1989.
- Great Lakes Health Effects Program. Reports of Health Data and Statistics for the Populations in Each of 17 Areas of Concern (1986–1992). Ottawa, ON: Great Lakes Health Effects Program, Health Canada, 1998.
- Carpenter DO. Human health effects of environmental pollutants: new insights. Environ Monit Assess 53:245–258 (1998).
- Wobeser GA. Diseases of Wild Waterfowl, 2nd ed. New York, NY: Plenum Press, 1997.
- Fox GA. What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. J Great Lakes Res 19:722–736 (1993).
- Anderson S, Sadinski W, Shugart L, Brussard P, Depledge M, Fort T, Hose J, Stegeman J, Suk W, Wirgin I, et al. Genetic and molecular ecotoxicology: a research framework. Environ Health Perspect 102(suppl 12):3–8 (1994).
- Peakall DB. Animal Biomarkers as Pollution Indicators. London: Chapman and Hall, 1992.
- Rose KA, Brewer LW, Barnhouse LW, Fox GA, Card NW, Mendonca M, Munkittrick KR, Vitt LJ. Ecological responses of oviparous vertebrates to contaminant effects on reproduction and development. In: Reproductive and Developmental Effects of Contaminants in Oviparous Vertebrates (DiGiulio RT, Tillitt DE, eds). Pensacola, FL: SETAC Press, 1999:225–281.
- Kleinow K, Baker J, Nichols J, Gobas F, Parkerton T, Muir D, Monteveddi G, Mastrodone P. Exposure, uptake, and disposition of chemicals in reproductive and developmental stages of selected oviparous vertebrates. In: Reproductive and Developmental Effects of Contaminants in Oviparous Vertebrates (DiGiulio RT, Tillitt DE, eds). Pensacola, FL: SETAC Press, 1999:9–111.
- Colborn TE. Epidemiology of Great Lakes bald eagles. J Toxicol Environ Health 33:395–453 (1991).
- Hebert CE, Norstrom RJ, Weseloh DV. A quarter century of environmental surveillance: the Canadian Wildlife Service's Great Lakes herring gull monitoring program. Environ Rev 7:147–166 (1999).
- Pekarik C, Weseloh DV. Organochlorine contaminants in herring gull eggs from the Great Lakes, 1974–1995: change point regression analysis and short-term regression. Environ Monit Assess 53:77–115 (1998).
- Grasman KA, Scanlon PF, Fox GA. Reproductive and physiological effects of environmental contaminants in fish-eating birds of the Great Lakes: a review of historical trends. Environ Monit Assess 53:117–145 (1998).
- Grasman KA, Fox GA, Scanlon PF, Ludwig JP. Organochlorine-associated immunosuppression in pre fledgling Caspian terns and herring gulls from the Great Lakes: an ecoepidemiological study. Environ Health Perspect 104(suppl 4):829–842 (1996).
- Rattner BA, Melancon MJ, Custer TW, Hothem RL, King KA, LeCaptain LJ, Spann JW, Woodin BR, Stegeman JJ. Biomonitoring environmental contamination with pipping black-crowned night heron embryos: induction of cytochrome P450. Environ Toxicol Chem 12:1719–1732 (1993).
- Hoffman DJ, Smith GJ, Rattner BA. Biomarkers of contaminant exposure in common terns and black-crowned night herons in the Great Lakes. Environ Toxicol Chem 12:1095–1103 (1993).
- Champoux L, DesGranges J-L, Rodrigue J, Hontela A, Trudeau S, Spear PE. Evaluation d'Indicateurs Biochimiques chez le Grand Heron, *Ardea herodias*, et le Bihoreau Gris, *Nycticorax nycticorax*, en Relation avec la Contamination du Fleuve Saint-Laurent. Serie de Rapports Techniques No. 354. Sainte Foy, Quebec: Region du Quebec, Service Canadien de la Faune, 2000.
- Champoux L, Ewins PJ, Struger J, Mineau P, Bishop CA, Postupalsky S, Ludwig JP. Double-crested cormorants of the Great Lakes: changes in population size, breeding distribution

- and reproductive output between 1913 and 1991. *Colon Waterbirds* 18(special publ 1):48–59 (1995).
24. Fox GA, Collins B, Hayakawa, Weseloh DV, Ludwig JP, Kubiak TJ, Erdman TC. Reproductive outcomes in colonial fish-eating birds: a biomarker for developmental toxicants in Great Lakes food chains. II: Spatial variation in the occurrence and prevalence of bill defects in young double-crested cormorants in the Great Lakes, 1979–1987. *J Gt Lakes Res* 17:158–167 (1991).
 25. Ludwig JP, Kurita-Matsuba H, Auman HJ, Ludwig ME, Summer CL, Giesy JP, Tillitt DE, Jones PD. Deformities, PCBs, and TCDD-equivalents in double-crested cormorants (*Phalacrocorax auritus*) and Caspian terns (*Hydroprogne caspia*) of the upper Great Lakes 1986–1991: testing the cause-effect hypothesis. *J Gt Lakes Res* 22:172–197 (1996).
 26. Yamashita N, Tanabe S, Ludwig JP, Kurita H, Ludwig ME, Tasukawa R. Embryonic abnormalities and organochlorine contamination in double-crested cormorants (*Phalacrocorax auritus*) and Caspian terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. *Environ Pollut* 79:163–173 (1993).
 27. Ryckman DP, Weseloh DV, Hamr P, Fox GA, Collins B, Ewins PJ, Norstrom RJ. Spatial and temporal trends in organochlorine contamination and bill deformities in double-crested cormorants (*Phalacrocorax auritus*) from the Canadian Great Lakes. *Environ Monit Assess* 53:169–195 (1998).
 28. Ludwig JP, Auman HJ, Kurita H, Ludwig ME, Campbell LM, Giesy JP, Tillitt DE, Jones P, Yamashita N, Tanabe S, et al. Caspian tern reproduction in the Saginaw Bay ecosystem following a 100-year flood event. *J Gt Lakes Res* 19:96–108 (1993).
 29. Mora MA, Auman HJ, Ludwig JP, Giesy JP, Verbrugge DA, Ludwig ME. Polychlorinated biphenyls and chlorinated insecticides in plasma of Caspian terns: relationships with age, productivity, and colony site tenacity in the Great Lakes. *Arch Environ Contam Toxicol* 24:320–331 (1993).
 30. Grasman KA, Fox GA. Associations between altered immune function and organochlorine contamination in young Caspian terns (*Sterna caspia*) from Lake Huron, 1997–1999. *Ecotoxicology* 10:101–114 (2001).
 31. Bishop CA, Brooks RJ, Carey JH, Ng P, Norstrom RJ, Lean DRS. The case for a cause-effect linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra s. serpentina*) from Ontario, Canada. *J Toxicol Environ Health* 33:521–547 (1991).
 32. Bishop CA, Ng P, Brooks RJ, Kennedy S, Stegeman JJ, Norstrom RJ. Environmental contamination and developmental abnormalities in eggs and hatchlings of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes-St. Lawrence River basin (1989–1991). *Environ Pollut* 99:1–14 (1998).
 33. Wren CD. Cause-effect linkages between chemicals and populations of mink (*Mustela vison*) and otter (*Lutra canadensis*) in the Great Lakes basin. *J Toxicol Environ Health* 33:549–585 (1991).
 34. Foley RE, Jackling SJ, Sloan RJ, Brown MK. Organochlorine and mercury residues in wild mink and otter: comparison with fish. *Environ Toxicol Chem* 7:363–374 (1988).
 35. Heaton SN, Bursian SJ, Giesy JP, Tillitt DE, Render JA, Jones PD, Verbrugge DA, Kubiak TJ, Aulerich RJ. Dietary exposure of mink to carp from Saginaw Bay, Michigan. I. Effects on reproduction and survival, and the potential risks to wild mink populations. *Arch Environ Contam Toxicol* 28:334–343 (1995).
 36. Mierle G, Addison EM, MacDonald KS, Joachim DG. Mercury levels in tissues of otters from Ontario, Canada: variation with age, sex, and location. *Environ Toxicol Chem* 19:3044–3051 (2000).
 37. Harding LE, Harris ML, Stephen CR, Elliott JE. Reproductive and morphological condition of wild mink (*Mustela vison*) and river otter (*Lutra canadensis*) in relation to chlorinated hydrocarbon contamination. *Environ Health Perspect* 107:141–147 (1999).
 38. Bishop CA, Mahony NA, Trudeau S, Pettit KE. Reproductive success and biochemical effects in tree swallows (*Tachycineta bicolor*) exposed to chlorinated hydrocarbon contaminants in wetlands of the Great Lakes and St. Lawrence River Basin, USA and Canada. *Environ Toxicol Chem* 18:263–271 (1999).
 39. Beland P, DeGuisse S, Girard C, Legace A, Martineau D, Michaud R, Muir DCG, Norstrom RJ, Pelletier E, Ray S, et al. Toxic compounds and health and reproductive effects in St. Lawrence beluga. *J Gt Lakes Res* 19:766–775 (1993).
 40. Martineau D, De Guise S, Fournier M, Shugart L, Girard C, Legace A, Beland P. Pathology and toxicology of beluga whales from the St. Lawrence Estuary, Quebec, Canada. Past, present and future. *Sci Total Environ* 154:201–215 (1994).
 41. Martineau D, Lair S, De Guise S, Lipscomb TP, Beland P. Cancer in beluga whales from the St. Lawrence Estuary, Quebec, Canada: a potential biomarker of environmental contamination. *J Cetacean Res Manag* (special publ 1):249–265 (1999).
 42. Moccia RD, Fox GA, Britton A. A quantitative assessment of the thyroid histopathology of herring gulls (*Larus argentatus*) from the Great Lakes and a hypothesis on the causal role of environmental contaminants. *J Wildl Dis* 22:60–70 (1986).
 43. Fox GA. Unpublished data.
 44. Fox GA, Trudeau S, Won H, Grasman KA. Monitoring the elimination of persistent toxic substances from the Great Lakes: chemical and physiological evidence from adult herring gulls. *Environ Monit Assess* 53:147–168 (1998).
 45. Moccia RD, Leatherland JF, Sonstegard RA. Quantitative inter-lake comparison of thyroid pathology in Great Lakes coho (*Oncorhynchus kisutch*) and chinook (*Oncorhynchus tshawytscha*) salmon. *Cancer Res* 41:2200–2210 (1981).
 46. Sonstegard RA, Leatherland JF. Hypothyroidism in rats fed Great Lakes coho salmon. *Bull Environ Contam Toxicol* 22:779–784 (1979).
 47. Villeneuve DC, Valli VE, Norstrom RJ, Freeman H, Sangalang GB, Ritter L, Becking GC. Toxicological response of rats fed Lake Ontario or Pacific coho salmon for 28 days. *J Environ Sci Health* 19:649–689 (1981).
 48. Beierwaltes WH. The most common thyroid disease in the State of Michigan is endemic goiter not due to iodine deficiency. *Washtenaw County Med Soc Bull* 39:3–10 (1987).
 49. Lorenzen A, Moon TW, Kennedy SW, Fox GA. Relationships between environmental organochlorine contaminant residues, plasma corticosterone concentrations, and intermediary metabolic enzyme activities in Great Lakes herring gull embryos. *Environ Health Perspect* 107:179–186 (1999).
 50. Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. *J Wildl Dis* 33:430–437 (1997).
 51. Hazelwood RL. Pancreas. In: *Sturkie's Avian Physiology*, 5th ed (Whitton GC, ed). San Diego, CA:Academic Press, 2000:539–555.
 52. Guha B, Gosh A. Diabetes and avian resistance. *Curr Sci* 62:564–568 (1992).
 53. Klasing KC. *Comparative Avian Nutrition*. New York, NY:CAB International, 1998:201–209.
 54. Poland A, Knutson JC. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol* 22:517–554 (1982).
 55. Peterson RE, Seefeld MD, Christian BJ, Potter CL, Kelling CK, Keesey RE. The wasting syndrome in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity: basic features and their interpretation. In: *The Banbury Report - Biological Mechanisms of Dioxin Action*. Cold Spring Harbor, NY:Cold Spring Harbor Laboratory, 1984:291–308.
 56. Gorski JR, Weber LWD, Rozman K. Reduced gluconeogenesis in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-treated rats. *Arch Toxicol* 64:66–71 (1990).
 57. Weber LWD, Lebofsky M, Greim H, Rozman K. Key enzymes of gluconeogenesis are -dose-dependently reduced in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-treated rats. *Arch Toxicol* 65:119–123 (1991).
 58. Weber LWD, Stahl BU, Lebofsky M, Alper RH, Kerecsen L, Rozman K. Inhibition of phosphoenolpyruvate carboxylase activity appears to be the key biochemical lesion in the acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Chemosphere* 23:1957–1962 (1991).
 59. Gilbertson M, Kubiak T, Ludwig J, Fox G. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. *J Toxicol Environ Health* 33:455–520 (1991).
 60. Kubiak TJ, Harris HJ, Smith LM, Schwartz TR, Stalling DL, Trick JA, Sileo L, Docharty D, Erdman TC. Microcontaminants and reproductive impairment of the Forster's tern on Green Bay, Lake Michigan - 1983. *Arch Environ Contam Toxicol* 18:706–727 (1989).
 61. Harris HJ, Erdman TC, Ankley GT, Lodge KB. Measures of reproductive success and polychlorinated biphenyl residues in eggs and chicks of Forster's terns on Green Bay, Lake Michigan, Wisconsin - 1988. *Arch Environ Contam Toxicol* 25:304–314 (1993).
 62. Fox GA, Kennedy SW, Norstrom RJ, Wigfield DC. Porphyria in herring gulls: a biochemical response to chemical contamination of Great Lakes food chains. *Environ Toxicol Chem* 7:831–839 (1988).
 63. Kennedy SW, Fox GA. Highly carboxylated porphyrins as a biomarker of polyhalogenated aromatic hydrocarbon exposure in wildlife: confirmation of their presence in Great Lakes herring gull chicks in the early 1970s and important methodological details. *Chemosphere* 21:407–415 (1990).
 64. Kennedy SW. *Studies on Porphyria as an Indicator of Polyhalogenated Aromatic Hydrocarbon Exposure* [PhD Thesis]. Ottawa, Canada:Department of Chemistry, Carleton University, 1988.
 65. Kennedy SW, Fox GA, Trudeau S, Bastien LJ, Jones SP. Highly carboxylated porphyrin concentrations; a biochemical marker for PCB exposure in herring gulls. *Mar Environ Res* 46:65–69 (1998).
 66. van Birgelen APJM, DeVito MJ, Akins JM, Ross DG, Diliberto JJ, Birnbaum LS. Relative potencies of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls derived from hepatic porphyrin accumulation in mice. *Toxicol Appl Pharmacol* 138:98–109 (1996).
 67. Grasman KA. *Immunological and Hematological Biomarkers for Contaminants in Fish-eating Birds of the Great Lakes* [PhD Thesis]. Blacksburg, VA:Virginia Polytech, 1995.
 68. Swain WR. Effects of organochlorine chemicals on the reproductive outcome of humans who consumed contaminated Great Lakes fish: an epidemiological consideration. *J Toxicol Environ Health* 33:587–639 (1991).
 69. Weisglas-Kuperus N, Patandin S, Berbers GAM, Sas TCJ, Mulder PGH, Sauer PJJ, Hooijkaas H. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. *Environ Health Perspect* 108:1203–1207 (2000).
 70. Barron MG, Anderson MJ, Cacula D, Lipton J, The SJ, Hinton DE, Zelikoff JT, Ditkeboom AL, Tillitt DE, Holey M, et al. PCBs, liver lesions, and biomarker responses in adult walleye (*Stizostedion vitreum vitreum*) collected from Green Bay, Wisconsin. *J Gt Lakes Res* 26:250–271 (2000).
 71. De Guise S, Martineau D, Beland P, Fournier M. Possible mechanisms of action of environmental contaminants on St. Lawrence beluga whales. *Environ Health Perspect* 103(suppl 4):73–77 (1995).
 72. Hartsough GR. Great Lakes fish now suspect as mink food. *Am Fur Breeder* 38:35–37 (1965).
 73. Gilbertson M. Epidemics in birds and mammals caused by chemicals in the Great Lakes. In: *Toxic Contaminants and Ecosystem Health; A Great Lakes Focus* (Evans MS, ed). New York:Wiley, 1988:133–152.
 74. Aulerich RJ, Ringer RK. Some effects of chlorinated hydrocarbon pesticides on mink. *Am Fur Breeder* 43:10–11 (1970).
 75. Hornshaw TC, Aulerich RJ, Johnson HE. Feeding Great Lakes fish to mink: effects on mink and accumulation and elimination of PCBs by mink. *J Toxicol Environ Health* 11:933–946 (1983).
 76. Haffner GD, Gloschenko V, Straughan CA, Hebert CE, Lazar R. Concentrations and distributions of polychlorinated biphenyls, including non-ortho congeners, in mink populations from southern Ontario. *J Gt Lakes Res* 24:880–888 (1998).
 77. Bowerman WW, Giesy JP, Best DA, Kramer VJ. A review of factors affecting productivity of bald eagles in the Great Lakes region: implications for recovery. *Environ Health Perspect* 103 (suppl 4):51–59 (1995).
 78. Grier JW. Ban on DDT and subsequent recovery of reproduction in bald eagles. *Science* 218:1232–1235 (1982).
 79. Summer CL, Giesy JP, Bursian SJ, Render JA, Kubiak TJ, Jones PD, Verbrugge DA, Aulerich RJ. Effects induced by feeding organochlorine-contaminated carp from Saginaw Bay, Lake Huron, to laying white leghorn hens. II: Embryotoxic and teratogenic effects. *J Toxicol Environ Health* 49:409–438 (1996).
 80. Bowerman WW, Kubiak TJ, Holt JB Jr, Evans DL, Eckstein RG, Sindelar CR, Best DA, Kozie KD. Observed abnormalities in mandibles of nestling bald eagles *Haliaeetus leucocephalus*. *Bull Environ Contam Toxicol* 53:450–457 (1994).
 81. Bishop CA, Gendron AD. Reptiles and amphibians: shy and sensitive vertebrates of the Great Lakes basin and St. Lawrence River. *Environ Monit Assess* 53:225–244 (1998).
 82. De Guise S, Lagace A, Beland P. True hermaphroditism in a St. Lawrence beluga whale (*Depphinapterus leucas*). *J Wildl Dis* 30:287–290 (1994).
 83. Fox GA. Epidemiological and pathobiological evidence of contaminant-induced alterations in sexual development in free-living wildlife. In: *Chemically-Induced Alterations in Sexual and Functional Development; The Wildlife/Human Connection* (Colborn T, Clement C, eds). Princeton, NJ:Princeton Science Publishing Co., 1992.
 84. Conover RM. Occurrence of supernormal clutches in Laridae. *Wilson Bull* 96:249–267 (1984).
 85. Conover RM, Hunt GL. Female-female pairing and sex ratios in gulls: a historical perspective. *Wilson Bull* 96:619–625 (1984).
 86. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist. *Nature* 375:581–585 (1995).

87. Monosson E, Kelce WR, Mac M, Gray LE. Environmental antiandrogens: potential effects on fish reproduction and development. In: Chemically Induced Alterations in Functional Development and Reproduction of Fishes (Rolland RM, Gilbertson G, Peterson RE, eds). Pensacola, FL:SETAC Press, 1995:53–60.
88. Kelly ME, Grasman KA, Fox GA. Gonadal Histology of Great Lakes Herring Gulls. Philadelphia, PA:SETAC, 1999.
89. De Solla SR, Bishop CA, van der Kraak G, Brooks RJ. Impact of organochlorine contamination on levels of sex hormones and external morphology of common snapping turtles (*Chelydra serpentina serpentina*) in Ontario, Canada. Environ Health Perspect 106:253–260 (1998).
90. Friend M, Franson JC. Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds. Information and Technology Report 1999-001. Washington, DC:U.S. Geological Survey, Biological Resources Division, 1999.
91. Siegfried LM. Neoplasms identified in free-living birds. Avian Dis 27:86–99 (1983).
92. Franson JC. Personal communication.
93. Campbell DM. Personal communication.
94. Scott JM. Personal communication.
95. Patenaude NJ, Quinn JS, Beland P, Kingsley M, White BN. Genetic variation of the St. Lawrence beluga whale population assessed by DNA fingerprinting. Mol Ecol 3:375–381 (1994).
96. Murdock MH, Hebert PDN. Mitochondrial DNA diversity of brown bullhead from contaminated and relatively pristine sites in the Great Lakes. Environ Toxicol Chem 13:1281–1289 (1994).
97. Harshbarger JC, Clark JB. Epizootiology of neoplasms in bony fish of North America. Sci Total Environ 94:1–32 (1990).
98. Baumann PC. The use of tumors in wild populations of fish to assess ecosystem health. J Aquat Ecosyst Health 1:135–146 (1992).
99. Baumann PC, Harshbarger JC, Hartman KJ. Relationship between liver tumors and age in brown bullhead populations from two Lake Erie tributaries. Sci Total Environ 94:71–87 (1990).
100. Baumann PC. Reduced PAH causes decreased liver tumors and increased life span in Black River bullhead. Abstract, SETAC-92, Cincinnati, OH.
101. Baumann PC, Harshbarger JC. Decline in liver neoplasms in wild brown bullhead catfish after closing plant closes and environmental PAHs plummet. Environ Health Perspect 103:168–170 (1995).
102. Yauk CL, Fox GA, McCarty BE, Quinn JS. Induced minisatellite germline mutations in herring gulls (*Larus argentatus*) living near steel mills. Mutation Res 452:211–218 (2000).
103. White PA, Robitaille S, Rasmussen JB. Heritable reproductive effects of benzo[a]pyrene on the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 18:1843–1847 (1999).
104. Jacobson JL, Jacobson SW, Fein GG, Schwartz PM, Dowler JK. Prenatal exposure to an environmental toxin: a test of the multiple effects model. Dev Psychol 20:523–532 (1984).
105. Lonky E, Reihman J, Darvill T, Mather J Sr, Daly H. Neonatal behavioral assessment scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. J Gt Lakes Res 22:198–212 (1996).
106. Daly HB. Laboratory rat experiments show consumption of Lake Ontario salmon causes behavioral changes: support for wildlife and human research results. J Gt Lakes Res 19:784–788 (1993).
107. Henshel DS, Martin JW, Norstrom RJ, Elliott J, Cheng KM, DeWitt JC. Morphometric brain abnormalities in double-crested cormorant chicks exposed to polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls. J Gt Lakes Res 23:11–26 (1997).
108. Henshel DS. Developmental neurotoxic effects of dioxin and dioxin-like compounds on domestic and wild avian species. Environ Toxicol Chem 17:88–98 (1998).
109. Fox GA, Gilman AP, Peakall DB, Anderka FW. Behavioral abnormalities of nesting Lake Ontario herring gulls. J Wildl Manag 42:477–483 (1978).
110. McArthur MLB, Fox GA, Peakall DB, Philogene JBR. Ecological significance of behavioral and hormonal abnormalities in breeding ring doves fed an organochlorine chemical mixture. Arch Environ Contam Toxicol 12:343–355.
111. Schrank CS, Cormier SM, Blazer VS. Contaminant exposure, biochemical, and histopathological biomarkers in white suckers from contaminated and reference sites in the Sheboygan River, Wisconsin. J Gt Lakes Res 23:119–130 (1997).
112. Bowerman WW, Best DA, Grubb TG, Zimmerman GM, Giesy JP. Trends of contaminants and effects in bald eagles of the Great Lakes basin. Environ Monit Assess 53:197–212 (1998).
113. Johnson BL, Hicks HE, De Rosa CT. Key environmental human health issues in the Great Lakes and St. Lawrence River basins. Environ Res A80(2 pt 2):S2–S12 (1999).
114. National Research Council. Animals as Sentinels of Environmental Hazards. Washington, DC:National Academy Press, 1991.
115. Gilbertson M, Fox G, Bowerman W. Designing the Environmental Results Workshop: historical context, causality and candidate species. Environ Monit Assess 53:17–55 (1998).
116. Johnson BL, De Rosa CT. Public health implications. Environ Res A80(2 pt 2):S246–S248 (1999).